

# **Molecular Predictors of 3D Morphogenesis by Breast Cancer Cells in 3D Culture**

*Ju Han*

**Imaging & Informatics Lab  
Life Sciences Division  
<http://vision.lbl.gov>**

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# Outline

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- Motivation
- Experimental design
- Previous work
- Approach
- Results
- Summary

# Motivation

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- A panel of cell lines for analysis
  - Introduce necessary molecular diversity
  - Generate heterogeneous responses to the treatment
  - Offer an improved model system for high-content screening, comparative analysis, and cell systems biology
- Morphometric subtyping for a panel of breast cancer cell lines in identifying
  - subpopulations with similar morphometric properties
  - molecular predictors for each subpopulations

# Experimental design



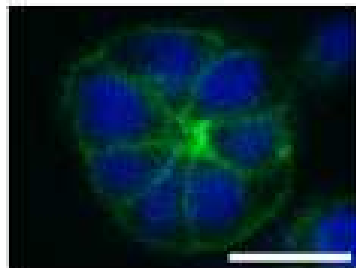
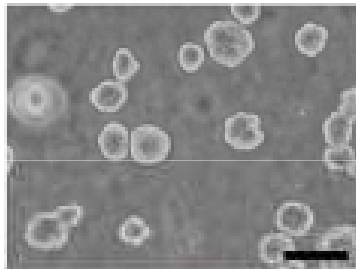
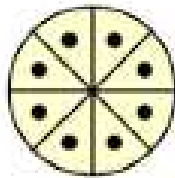
- A panel of 24 breast cancer cell lines
  - 600MPE, AU565, BT474, BT483, BT549, CAMA1, HCC1569, HCC70, HS578T, MCF12A, MCF7, MDAMB231, MDAMB361, MDAMB415, MDAMB436, MDAMB453, MDAMB468, S1, SKBR3, T4, T47D, UACC812, ZR751, ZR75B
- All 3D cell cultures were maintained for 4 days with media change every 2 days, and samples were then imaged with phase contrast microscopy
- Computational pipeline
  - Colony segmentation and representation
  - Phenotypic clustering
  - Molecular predictor of morphometric clusters
  - Molecular predictor of morphometric features

# Previous work

(Kenny et. al, Gene Ontology, 2007)

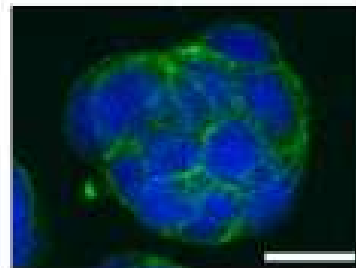
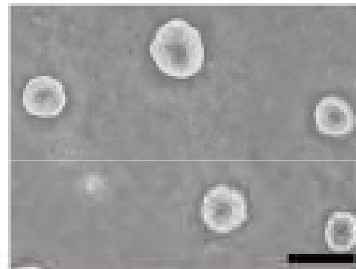
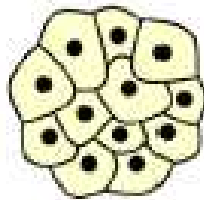


Round



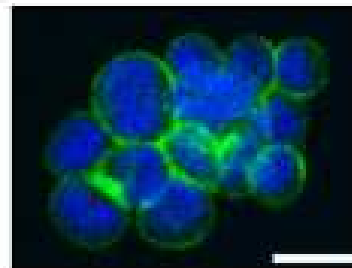
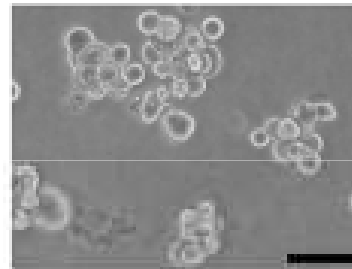
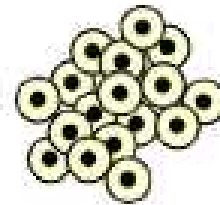
- Organized nuclei
- Robust cell-cell adhesion

Mass



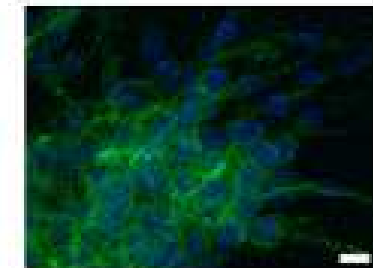
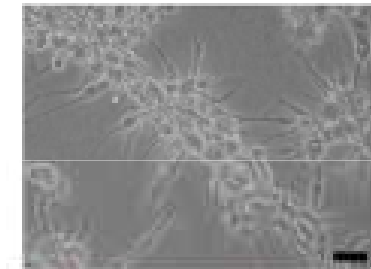
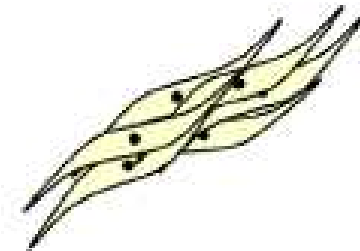
- Disorganized nuclei
- Robust cell-cell adhesion

Grape-like



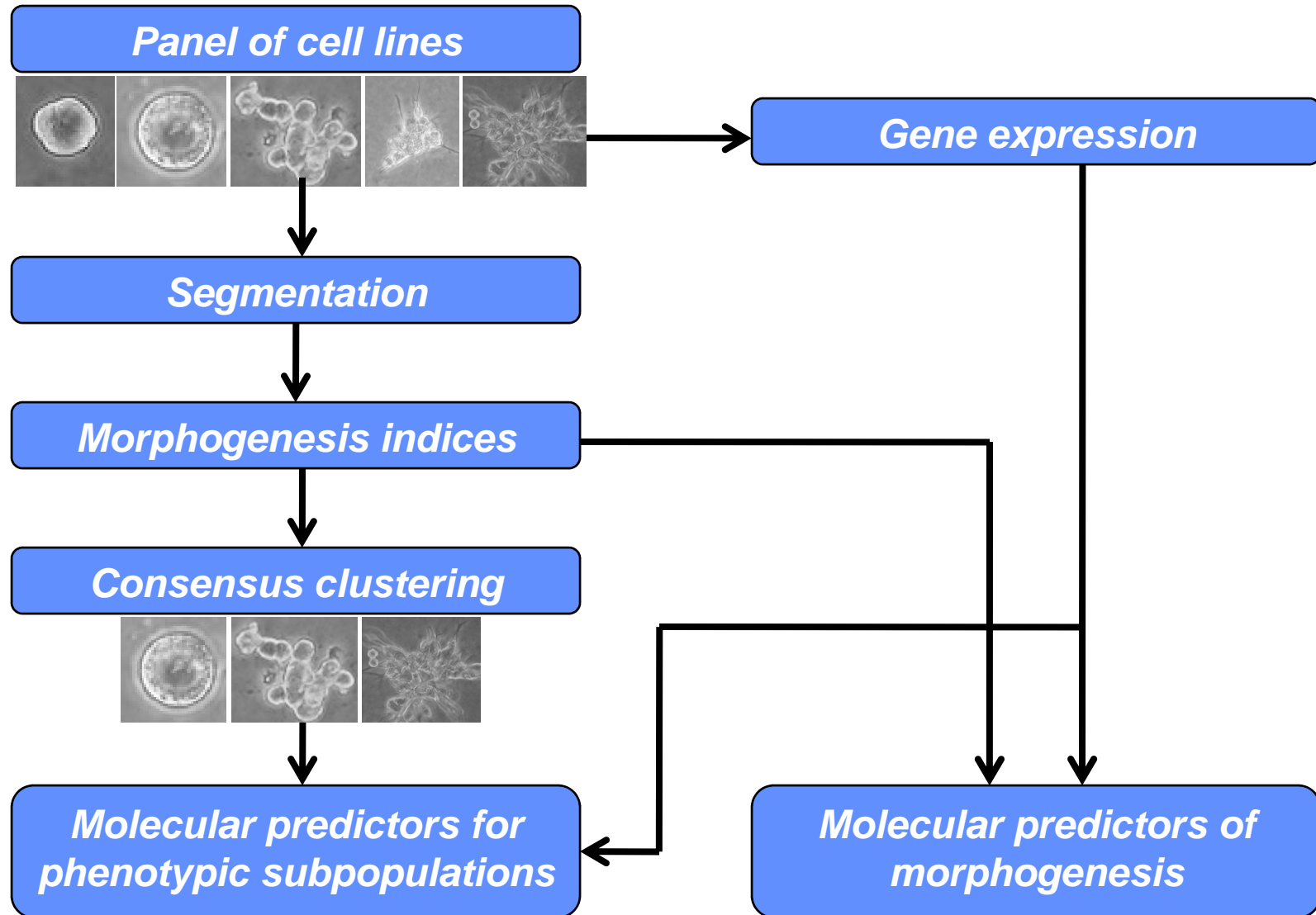
- Disorganized nuclei
- Poor cell-cell adhesion

Stellate



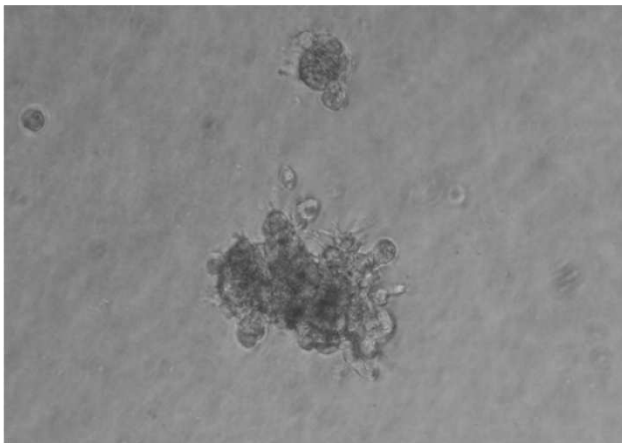
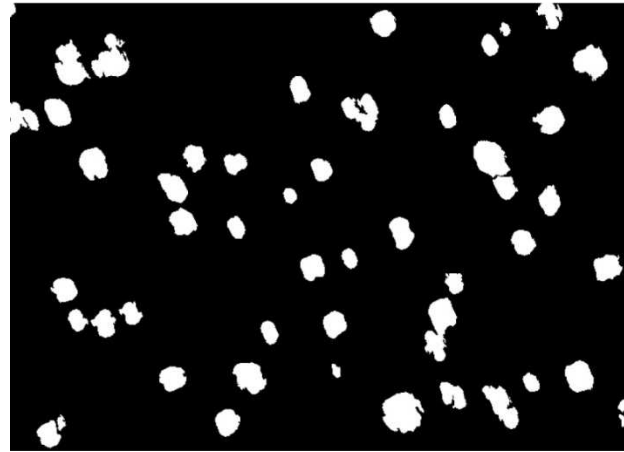
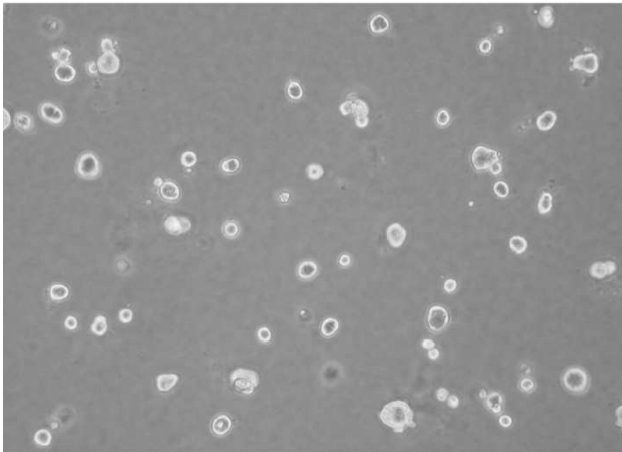
- Disorganized nuclei
- Elongated cell body with invasive processes

# Automatic subtyping a panel of breast cancer cell lines in 3D culture



# Colony segmentation and representation (phase images)

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Colonies are separated from the background based on texture features;  
Morphometric features (size and shape) are extracted for each colony.

# Clustering of morphometric features

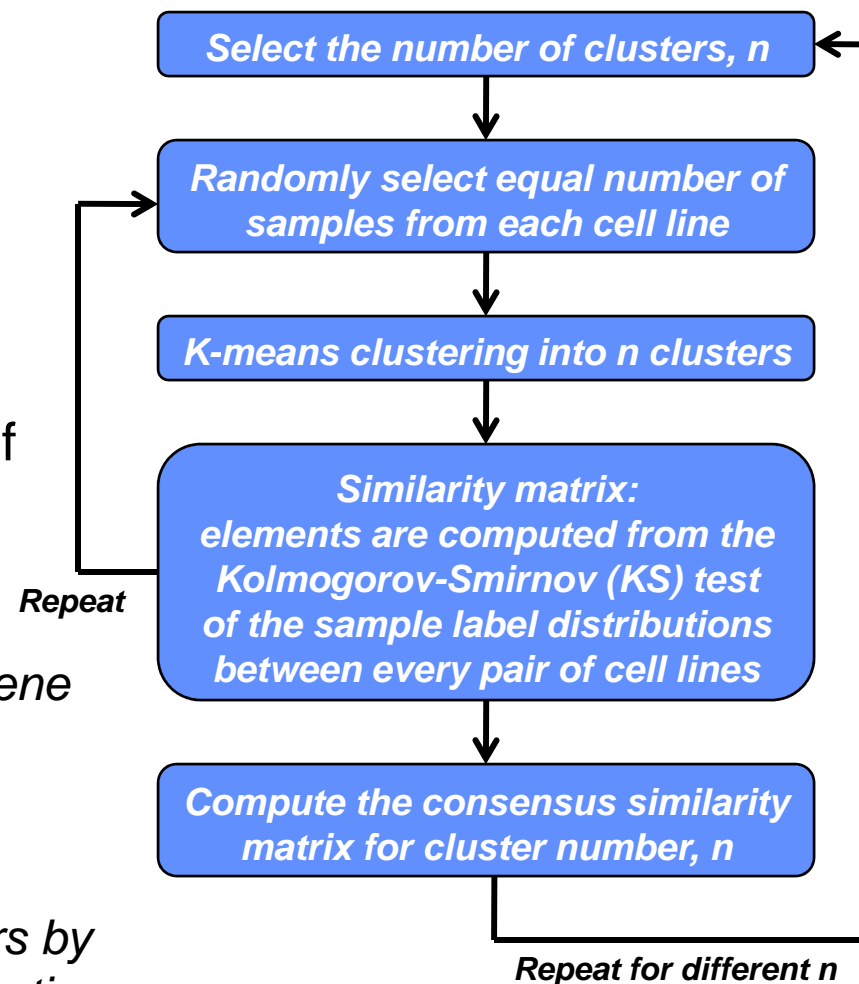


- Challenges

- Morphometric features are heterogeneous for the same cell line
- Sample size varies for different cell lines
- there is no prior knowledge of the number of clusters

- Consensus clustering

- *A proven method in analyzing gene expression data (Monti et. al, Machine Learning 2003)*
- *Repeated random resampling*
- *Determine the number of clusters by evaluating the consensus distribution for different cluster numbers*

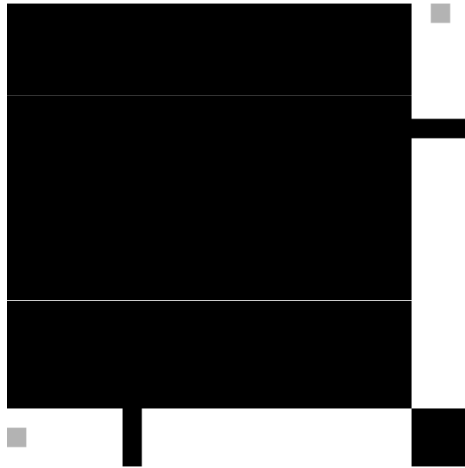




# Consensus clustering on a panel of 24 breast cancer cell lines in 3D



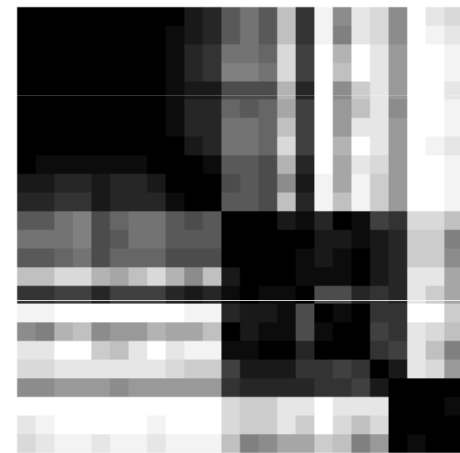
$N=2$



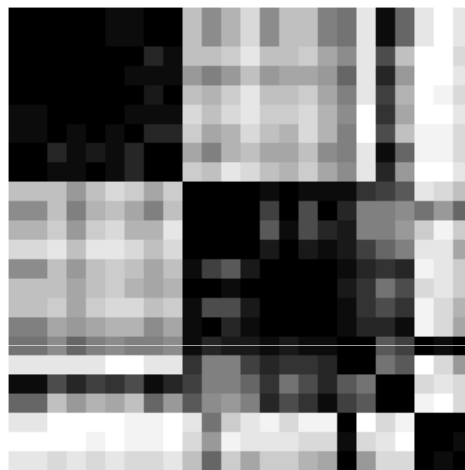
$N=3$



$N=4$

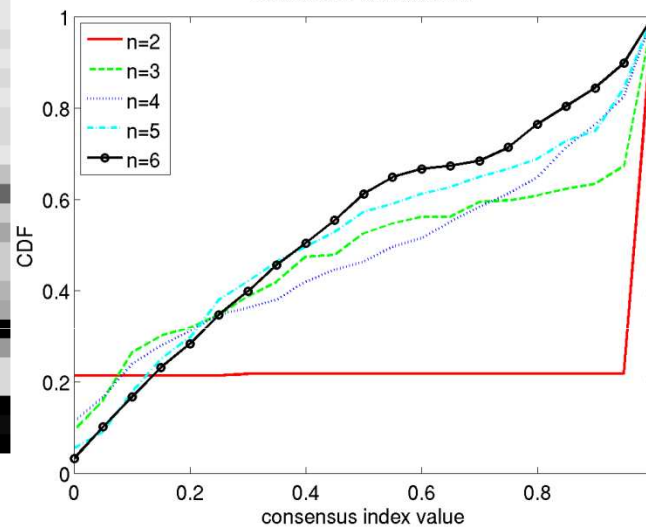


$N=5$



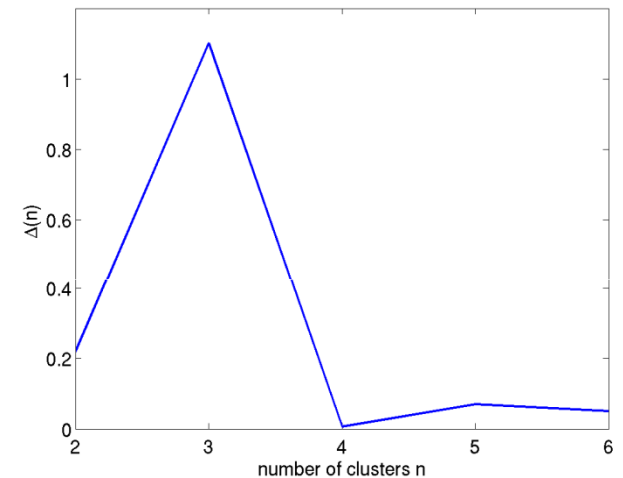
**CDF**

Consensus Matrix CDFs



**%change of CDF area**

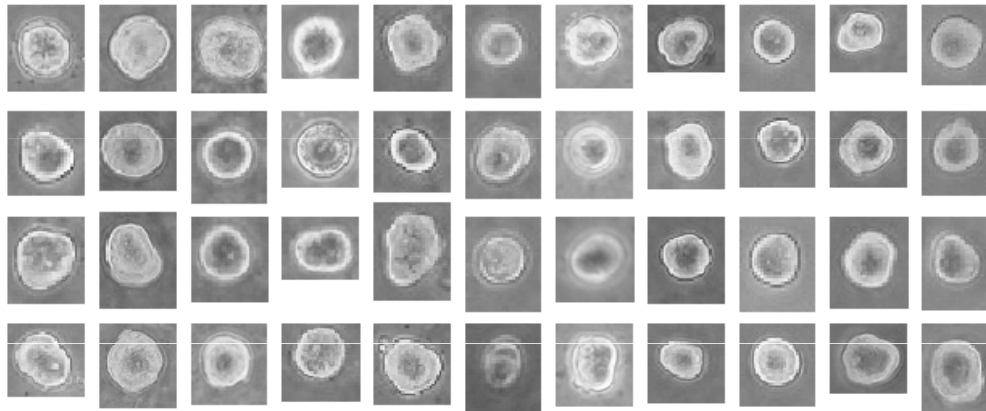
Change of area under CDFs



# Results with three clusters



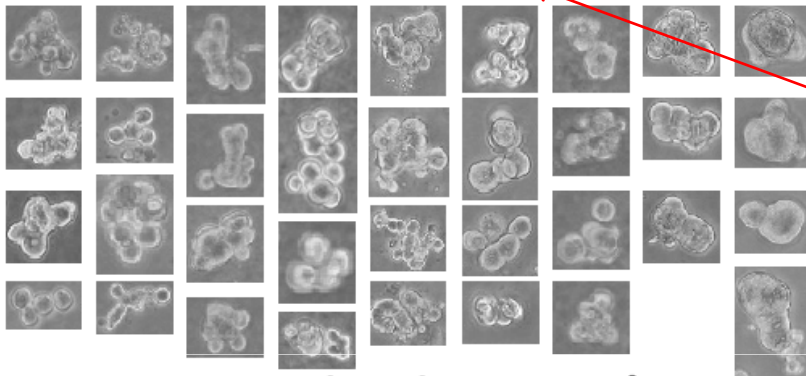
Round



MPE600 BT474 BT483 HCC1569 HCC70 MCF12A MCF7 MDAMB 415 S1 T4 T47D

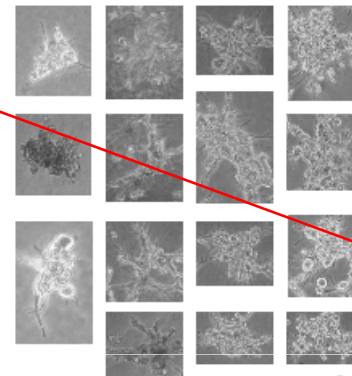
Consistent with previous manual clustering results (Kenny et. al, Gene Ontology, 2007)

Grape-like



AU565 CAMA1 MDAMB 361 MDAMB 453 MDAMB 468 SKBR3 UACC812 ZR751 ZR75B

Stellate



BT549 HS578T MDAMB 231 MDAMB 436

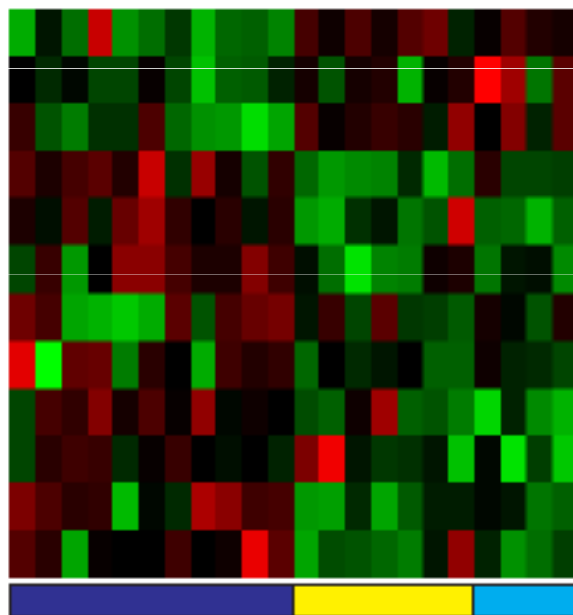
All triple-negative:  
estrogen receptors,  
progesterone  
receptors, and HER2

8 out of 9 cell lines  
express high levels of  
ERBB2

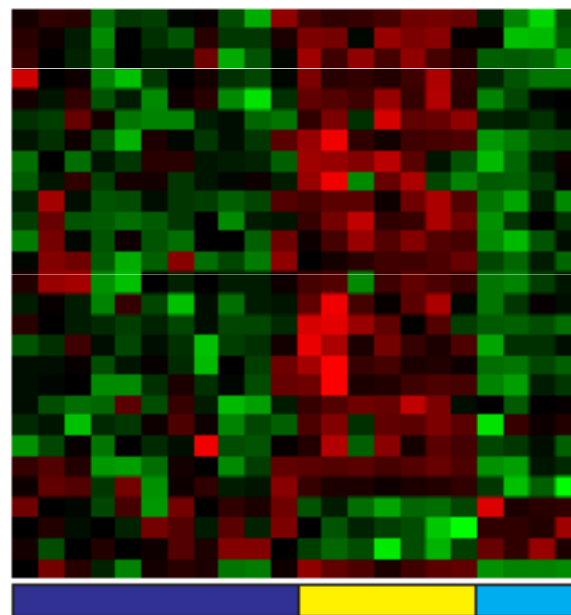
# Molecular predictors of morphometric clusters



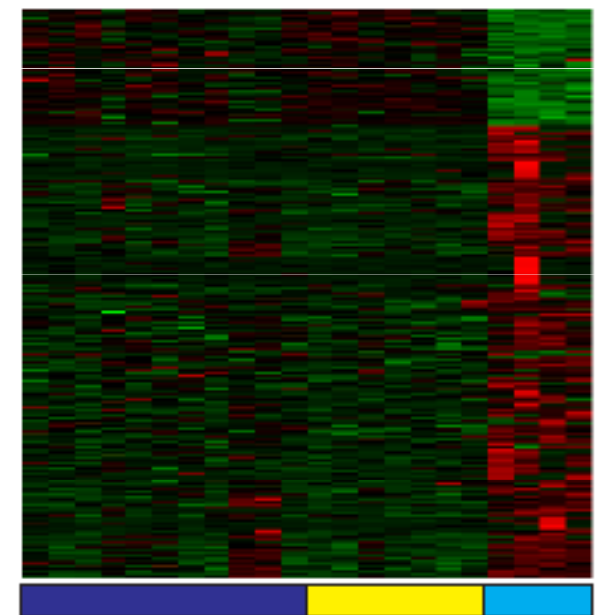
- Heat maps of top selected genes that best predict each of the three morphometric clusters
  - Gene ranking based on moderated t-test



Round



Grape-like



Stellate

# Best genes for predicting the stellate cluster



*implicated in the pathway of many diseases including cancer*

*affects the epithelial-mesenchymal transition in cancer*

Gene symbol	Gene description	$E_{B.632+}$	Expression level
PPARG	peroxisome proliferator-activated receptor gamma	0	+
FADS1///FADS3	fatty acid desaturase 1///fatty acid desaturase 3	0	+
ZEB1	zinc finger E-box binding homeobox 1	0.0013	+
PVRL3	poliovirus receptor-related 3	0.0024	+
AKAP2///PALM2///PALM2-AKAP2	A kinase (PRKA) anchor protein 2///paralemmin 2///PALM2-AKAP2	0.0036	+
DOCK10	dedicator of cytokinesis 10	0.0037	+
CLCN6	chloride channel 6	0.0043	+
CTAGE4///LOC100142659///LOC441294	similar to CTAGE6///CTAGE family, member 4///CTAGE family mem-	0.0047	-
DAB2	disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)	0.0048	+
FLJ10357	hypothetical protein FLJ10357	0.0063	+
PALM2-AKAP2	PALM2-AKAP2	0.0095	+
LEPRE1	leucine proline-enriched proteoglycan (leprecan) 1	0.0113	+
PRR5	proline rich 5 (renal)	0.0124	-
LOC100142659	CTAGE family member	0.0149	-
FBXL11	F-box and leucine-rich repeat protein 11	0.0169	-
PPP2R4	protein phosphatase 2A activator, regulatory subunit 4	0.0204	-
CDS1	CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 1	0.0244	-
F11R	F11 receptor	0.0245	-
DCBLD2	discoidin, CUB and LCCL domain containing 2	0.0252	+
HOOK2	hook homolog 2 (Drosophila)	0.0260	-

*affects cell morphogenesis involved in differentiation*

# Molecular predictors of morphometric features (colony size)



- Nonlinear correlation (logistic  $y = \frac{1}{1 + e^{-(\alpha + \beta x)}}$ )

Gene symbol	Gene description	r	p-value
→ PPARG	peroxisome proliferator-activated receptor gamma	0.8667	< 0.001
LPIN2	lipin 2	0.8450	< 0.001
VCL	vinculin	0.8145	< 0.001
CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	0.7970	< 0.001
RFTN1	raftlin, lipid raft linker 1	0.7960	< 0.001
PRR3	proline rich 3	0.7940	< 0.001
INSIG1	insulin induced gene 1	0.7932	< 0.001
APBA2	amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)	0.7884	< 0.001
CCDC99	coiled-coil domain containing 99	0.7547	< 0.001
SEC23A	Sec23 homolog A (S. cerevisiae)	0.7530	< 0.001
CYR61	cysteine-rich, angiogenic inducer, 61	0.7530	< 0.001
CLCN6	chloride channel 6	0.7527	< 0.001
AKAP2///PALM2///PALM2-AKAP2	A kinase (PRKA) anchor protein 2///paralemmin 2///PALM2-AKAP2	0.7505	< 0.001
FADS1///FADS3	fatty acid desaturase 1///fatty acid desaturase 3	0.7504	< 0.001
DOCK10	dedicator of cytokinesis 10	0.7483	< 0.001
MAP1B	microtubule-associated protein 1B	0.7471	< 0.001
TGFB2	transforming growth factor, beta 2	0.7459	< 0.001
ASB1	ankyrin repeat and SOCS box-containing 1	0.7406	< 0.001
SNAPC1	small nuclear RNA activating complex, polypeptide 1, 43kDa	0.7283	< 0.001
GFPT2	glutamine-fructose-6-phosphate transaminase 2	0.7264	< 0.001



# Discussion



- The gene expression profiles of the stellate colonies are the most distinct from the other two morphometric classes
- PPAR-gamma
  - A druggable target, and a hub for lipid metabolism
  - A nuclear receptor protein, functions as transcription factors, and can be spliced in multiple forms
  - A potent inducer of epithelial mesenchymal transition in intestinal epithelial cells
  - Involved in proliferation and differentiation
  - Shown to be highly expressed in metastasized human breast tissue

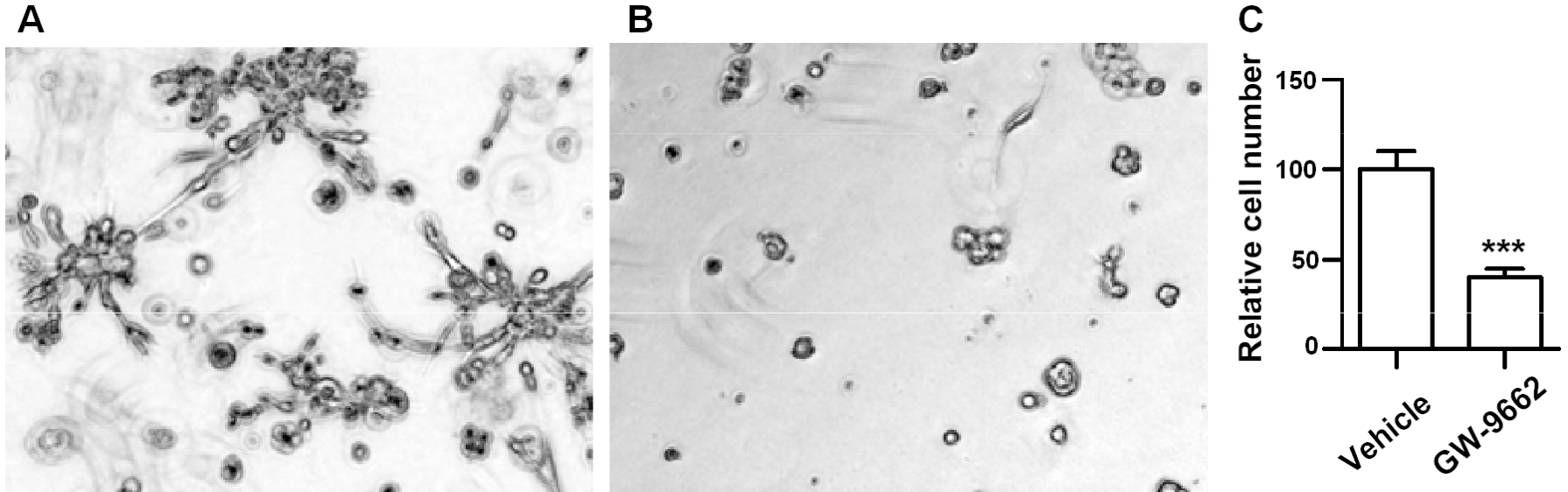
# Validation 1: *In vitro* experiment on PPARG

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- MDAMB231 was assayed in 3D cell cultures maintained in H14 medium with 1% fetal bovine serum
- The 3D cultures were prepared in triplicate by seeding single cells on top of a thin layer of Matrigel at a density of 2200 cells/cm<sup>2</sup> and overlaid by 5% final Matrigel diluted in culture medium
- **GW9662**, a PPARG inhibitor, was dissolved in DMSO and added to the 3D cultures in the final concentration of 10  $\mu$ M at the time of seeding
- The vehicle control was pure DMSO
- The culture medium and the drug were changed every other day
- Five images per well were collected after five full days in 3D culture

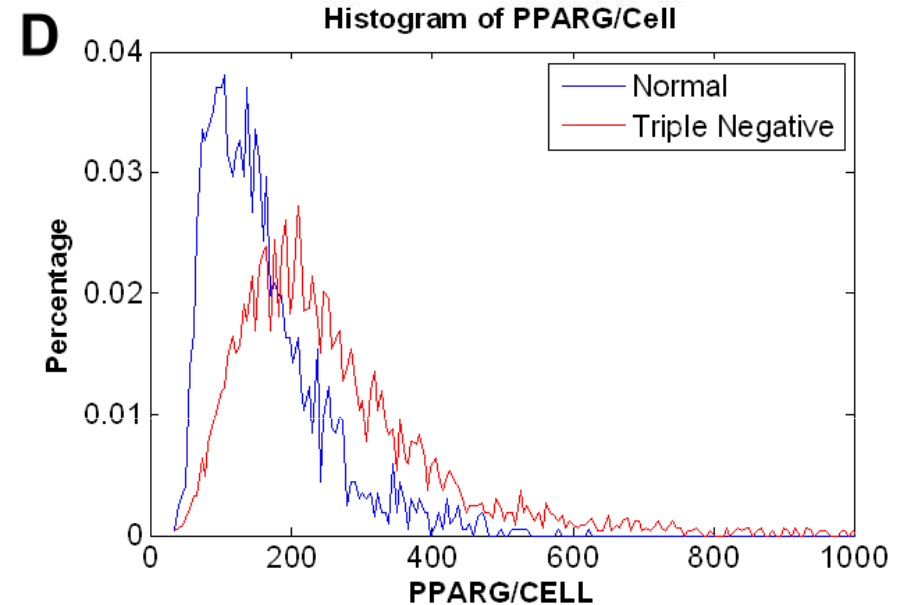
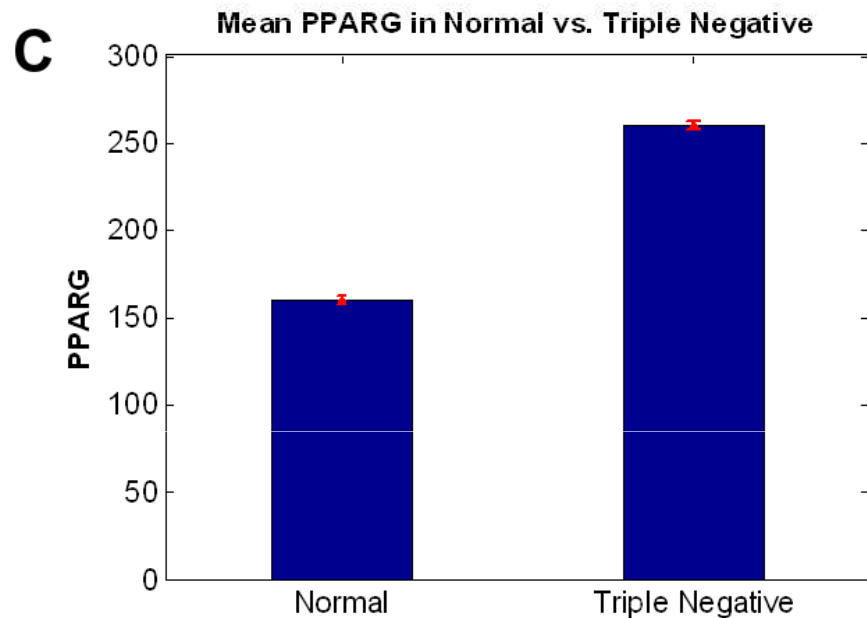
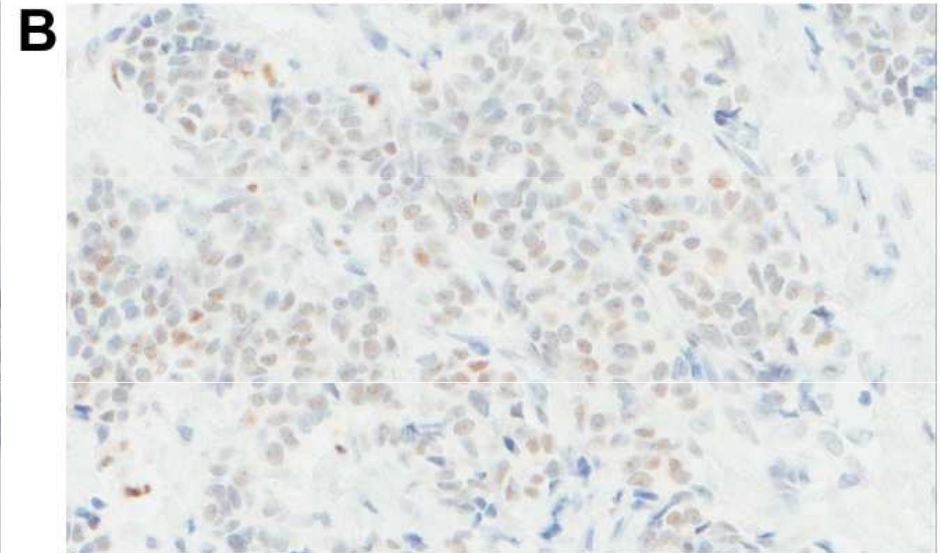
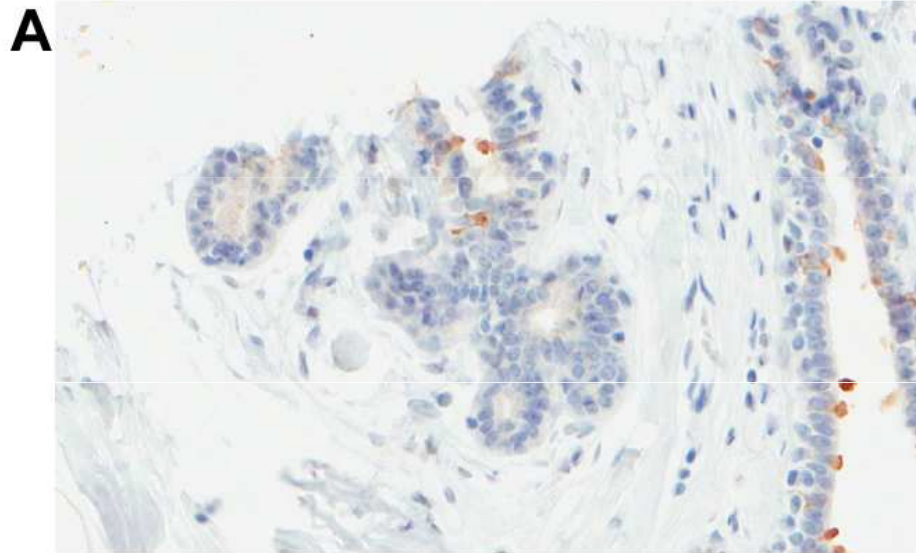
# *In vitro* validation results



- Treatment of a MDA-MB-231 with a PPARG-inhibitor indicates reduction in the proliferation rate: (A) untreated line, (B) treatment with Gw-9662, and (C) Proliferation index.
- The proliferation index was determined by incubating cultures with cell proliferation analysis reagent, WST1, on Day 5.



# Validation 2: *In vivo* experiment on PPARG



# Summary



- A system for identifying sub-populations for a panel of breast cancer cell lines
- These subpopulations are shown to compare well with previously manual clustering of the same data
- Robust statistics in
  - identifying those genes that differentiated computed sub-populations
  - determining genes that track with a specific morphometric feature
- Associative studies indicated that PPAR-gamma, a druggable target, correlates with the colony size and is highly expressed in the stellate subpopulation
- To appear in *PLoS Computational Biology*

# Acknowledgement

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  - Mina Bissell
- **Joe Gray**

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